A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

NeoBiota 102: 151–171 (2025)
DOI: 10.3897/neobiota.102.148301

Research Article

Shift in parasite load in native and non-native Eupercarian fish species living in sympatry

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Abstract

Non-native species have the potential to alter host community structure, which, in turn, may affect parasite transmission, diversity and distribution, particularly when the introduced host is ecologically or phylogenetically related to its native host(s). In this study, we examine whether the introduction of a competent non-native fish host shifts parasite load and community composition in native fish species. Specifically, we focus on two Eupercarian species: the native Eurasian perch (*Perca fluviatilis*) and the non-native pumpkinseed sunfish (*Lepomis gibbosus*), which was introduced into Europe from North America. Our results show that the parasite community of native perch co-occurring with non-native pumpkinseed (sympatric populations) differ significantly from those of perch inhabiting sites unaffected by pumpkinseed introduction (allopatric populations). Moreover, sympatric perch populations exhibit significantly higher parasite abundance, infracommunity richness and dominance, along with lower equitability, compared to allopatric populations. These findings suggest that the introduction of pumpkinseed may have contributed to shifts in both parasite abundance and diversity in native fish hosts. Additionally, our study indicates a potential parasite spillback of the trematode *Bucephalus polymorphus* and spill-over of the Asian invasive parasitic copepod *Neoergasilus japonicus*, both associated with pumpkinseed establishment in the study area. The successful inclusion of non-native fish species into the life cycles of several independently introduced and unrelated invasive parasites underscores the importance of introduced hosts in driving changes within local parasite communities.

Key words: Freshwater fish, parasite community, perch, pumpkinseed, species introduction, spill-back

OPEN ACCESS

Academic editor: Filipe Ribeiro
Received: 30 January 2025
Accepted: 16 April 2025
Published: 7 October 2025

Citation: Tkachenko MYu, Hnilička M, Janáč M, Kvach Yu, Vetešník L, Ondračková M (2025) Shift in parasite load in native and non-native Eupercarian fish species living in sympatry. In: Anastácio P, Ribeiro F, Chainho P (Eds) Invasions in Aquatic Systems. NeoBiota 102: 151–171. https://doi.org/10.3897/neobiota.102.148301

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Introduction

The introduction of non-native freshwater fish species has increased significantly across the world over the past few decades, leading to negative socio-economic, ecological and evolutionary impacts on local biodiversity (Hulme 2009; Vitule et al. 2009; Gozlan et al. 2010; Cucherousset and Olden 2011). Local ecosystems may be affected by invasive species through a range of mechanisms, including predation, competition for food and space, hybridisation, alterations in trophic relationships and modification of biochemical cycles (Leunda 2010; Cucherousset and Olden 2011). The success of an invasive species may also be partially linked to parasites and pathogens. For example, introduced species can gain an advantage over native species due to the absence of their

natural parasites (the so-called enemy release hypothesis; Keane and Crawley (2002)) or through the introduction of invasive parasites or pathogens that are more harmful to native competitors (Lymbery et al. 2014). While the role of parasites in the success of invasive species is widely recognised (e.g. Torchin et al. (2003); Prenter et al. (2004); Dunn (2009)), the impact of invasive species on parasite communities in native hosts has received less attention (Hohenadler et al. 2019).

Non-native species can alter local parasite communities through the introduction of non-native parasites capable of parasitising local hosts or by interacting with native parasite species. New parasite species co-introduced to the invaded ecosystem may either be host-specific and remain part of the parasitic fauna of their original host (Lymbery et al. 2014) or they may infect native species (Taraschewski 2006), potentially resulting in subsequent changes to local parasite diversity. For example, the introduction of Eurasian perch (*Perca fluviatilis*; Eupercaria, Percidae) into African lakes led to the co-introduction of several cestodes that later became established in native fish populations (Loker and Hofkin 2015). In France, monogenean parasites of the invasive common nase (*Chondrostoma nasus*) infected the threatened native south-west European nase (*Parachondrostoma toxostoma*) at sites where the two species co-occurred (Šimková et al. 2012). Following a successful introduction, therefore, non-native parasite species may be expected to transmit from the invasive species to native hosts, typically those that are ecologically similar or taxonomically close (Mack et al. 2000). This has led to the concept of "parasite spill-over" (Cleveland et al. 2002; Prenter et al. 2004), which suggests that non-native parasites can infect native host species in invaded habitats, thus accelerating their range expansion (Galli et al. 2005). Furthermore, these interactions may benefit the introduced host if the parasite negatively affects its new host (Tompkins et al. 2003).

However, interactions between non-native hosts and local parasite fauna appear to occur more frequently than the above-mentioned spill-over (Kelly et al. 2009). In this context, Kelly et al. (2009) introduced the concept of "parasite spill-back", which suggests that, when a non-indigenous species serves as a competent host for a native parasite, the presence of the additional host can amplify disease impacts on native species, affecting both individual hosts and entire populations. For example, in German rivers affected by the invasion of Ponto-Caspian species (gobiids and amphipods), local fish species exhibited higher infection rates by the nematode *Raphidascaris acus*, which successfully used Ponto-Caspian gobies as paratenic hosts, compared to rivers that had not been invaded (Hohenadler et al. 2019). A specific case of parasite spill-back is the hypothesis of "invasional meltdown" (Simberloff and von Holle 1999), which proposes that multiple species simultaneously colonising a new environment can enhance each other's chances of invasion. Certain ecological relationships that may develop amongst invasive species, such as predator-prey interactions, symbiosis and parasitism, could facilitate the establishment of invader parasites, even if the parasites were introduced through other vectors (Taraschewski 2006; Emde et al. 2014). This is particularly relevant for parasites with complex life cycles as their invasion success depends on the availability of suitable intermediate hosts. Interestingly, invasional meltdown may also occur within parasite-parasite relationships, as exemplified by the eel nematode *Anguillicola crassus*, which escapes the host's immune response by invading the cyst of another invasive parasite, the acanthocephalan *Pomphorhynchus laevis* (Hohenadler et al. 2018).

Non-native species can also alter local parasite communities through the "dilution effect". In this case, if they are not competent hosts for the acquired parasites,

non-native species may act as parasite sinks, or dilution agents, reducing parasite prevalence in local populations (Keesing et al. 2006; Gendron and Marcogliese 2017). Non-native species can also disrupt parasite transmission pathways. For example, fish can decrease the prevalence of trematodes in native fish populations by preying on the parasite's intermediate hosts, such as snails (Poulin and Mouillot 2003). Additionally, native specialist parasites may decline if non-native hosts replace native hosts, particularly when the non-native species are unsuitable hosts, potentially leading to the extinction of certain native parasite species (Hohenadler et al. 2019). This can result in the homogenisation of parasite communities, favouring generalist parasites (Dunn 2009). Finally, changes in host availability and community structure can shift competitive dynamics amongst parasite species, for example, when a diverse native host community is supplanted by a dominant non-native species, following which parasite diversity is reduced, promoting the dominance of a few generalist parasite species (Gendron et al. 2012).

Aquatic ecosystems are highly vulnerable to the introduction of non-native species, whether as hosts, such as fish, molluscs or crustaceans (Bódis et al. 2012) or their associated parasites (Esposito et al. 2023). Furthermore, the introduction of species into freshwater ecosystems can have numerous impacts on native communities and on ecosystem functioning (Havel et al. 2015). The Eurasian perch is a widely distributed predatory freshwater species (Stepien and Haponski 2015) inhabiting both lentic and lotic waterbodies (Baruš and Oliva 1995). It frequently co-occurs with the non-native pumpkinseed sunfish (*Lepomis gibbosus*; Eupercaria, Centrarchidae), a North American freshwater species (Scott and Crossman 1973) introduced into Europe as an ornamental fish in the late 19th century which has had negative impacts on local ecosystems (Soes et al. 2011). The pumpkinseed is now widespread across many European countries and was added to the list of Invasive Species of Union Concern in 2019 (European Commission 2019).

In its native North American range, the pumpkinseed commonly occurs sympatrically with the yellow perch (*Perca flavescens*), a sister species of the Eurasian perch (Collette and Banarescu 1977; Shcherbukha 1993), with both species sharing similar habitat and food preferences (Sun and Harvey 1986; Runciman and Leaf 2009). Despite millions of years of isolation, the parasite assemblages of these two perch species, composed of non-host-specific parasites, are not random, but contain key components that are predictable at the continental scale (Carney and Dick 1999). As a result, pumpkinseed in Europe share several common parasites with the Eurasian perch, such as the cestodes *Triaenophorus nodulosus* and *Bothriocestus claviceps* and the nematode *Camallanus lacustris* (Carney and Dick 1999; Masson et al. 2015; Ondračková et al. 2019a; Kvach et al. 2021a).

Building on present knowledge of the sympatric occurrence of these two Eupercarian fish species, this study aims to evaluate potential changes in parasite communities between populations where either perch or pumpkinseed live without their Eupercarian relative (allopatric sites) and populations where both species co-exist (sympatric sites). In doing so, we: 1) compare the abundance and species diversity of parasite communities to assess the ability of non-native pumpkinseed to acquire local parasite species, 2) evaluate the similarity of parasite communities between sympatric and allopatric populations in both host species and identify parasite species that significantly contribute to shifts in the parasite community of perch and 3) focus on parasite species shared between the perch and pumpkinseed and discuss their relevance in the context of potential parasite spill-back, spill-over, dilution or invasional meltdown.

Material and methods

Fish and parasite sampling

Fish were collected from eight small lentic waterbodies (oxbows and sand pits) in the lower Morava River Basin in the Czech Republic, three sites having allopatric populations of perch and of pumpkinseed (six sites in total) and two sites having sympatric populations of both species (Table 1). Fish were collected using electrofishing gear (SEN, fa Bednář R., Olomouc, Czech Republic; frequency 75–85 Hz; maximum output 225/300 V) or seine nets (7 m long, 1 cm mesh size), depending on habitat type. After collection, the fish were transported alive in aerated river water to the facilities at the Institute of Vertebrate Biology, where they were placed in outdoor tanks, separated by fish species and locality. Fifteen fish per species were collected for each site (75 perch and 75 pumpkinseed in total) and examined for parasites (Table 1). Following humane dispatch, standard length (SL, mm) was measured for each fish, after which the fins, skin, gills, gill covers, eyes, muscle and internal organs (intestine, liver, spleen, heart, kidney, gonads) were inspected for the presence of parasites. All fish were dissected within three days of sampling to ensure maximum parasite recovery (Kvach et al. 2016).

Table 1. List of sites sampled for parasite communities of native European perch *Perca fluviatilis* and non-native pumpkinseed sunfish *Lepomis gibbosus* in the Basin of the lower Morava River, Czech Republic, showing number of fish dissected (n), fish standard length (SL in mm (mean, range)), parasite prevalence (%), total abundance of parasites and parasite species richness.

Locality name	Coordinates	Fish species	n	Fish SL	Parasite prevalence	Parasite abundance	Parasite species richness
Sympatric sites							
Babice oxbow	49.117968, 17.488028	*P. fluviatilis*	15	69 (61-98)	100	209	12
		L. gibbosus	15	74 (63-81)	100	532	9
U Jezu sandpit	49.131407, 17.510408	*P. fluviatilis*	15	71 (63-79)	100	358	18
		L. gibbosus	15	75 (65-82)	100	989	10
Allopatric sites							
Dedava sandpit	48.633176, 16.959805	*P. fluviatilis*	15	71 (56-114)	100	123	10
Kanada oxbow	49.102925, 17.492513	*P. fluviatilis*	15	71 (63-76)	100	129	11
Penne oxbow	49.156364, 17.511674	*P. fluviatilis*	15	71 (66-87)	93	118	11
Dyje oxbow D3	48.684344, 16.916031	*L. gibbosus*	15	68 (52-84)	100	6623	4
Dyje oxbow D6	48.717369, 16.888908	*L. gibbosus*	15	59 (55-64)	100	97	5
Pohansko oxbow	48.725360, 16.890936	*L. gibbosus*	15	60 (53-70)	100	285	8

Parasite processing

Non-metazoan parasites were examined alive under light microscopy and their presence/absence recorded. Numbers of *Ichthyophthirius multifiliis* and *Dermocystidium* were recorded. Metazoan parasites were collected, counted and preserved for species identification. Monogeneans and myxosporeans were preserved in GAP (glycerine-ammonium-picrate) and prepared as semi-permanent slides (Malmberg 1970), while cestodes, trematodes, acanthocephalans and nematodes were preserved in hot 4% formaldehyde. Prior to species identification, cestodes and trematodes were stained with iron acetic carmine, dehydrated in ethanol of increasing concentration and mounted in Canada balsam as permanent slides (Georgiev et al. 1986; Cribb and Bray 2010). Acanthocephalans were pressed between two slides and preserved in 70% ethanol. Both preserved acanthocephalans and nematodes were mounted in glycerol as temporary slides for light microscopy. Crustaceans and bivalves were

preserved in 4% formaldehyde and identified under light microscopy. All preserved parasites were identified to species level, or to the lowest possible taxonomic level when species-level identification was not feasible. An Olympus BX53 light microscope (Olympus Optical Co., Hamburg, Germany) equipped with phase-contrast, differential (Nomarski) interference contrast and image analysis software (CellSens Standard digital image analysis package; Olympus Optical Co., Hamburg, Germany) was used for species identification. Identification followed the keys of Bauer (1987), Beverley-Burton (1984), Moravec (2013) and Sudarikov et al. (2002).

A subsample of larval cestodes (Bothriocephalidae, Proteocephalidae) and trematodes (*Diplostomum*), for which species identification could not be determined morphologically, was preserved in 96% ethanol for further molecular analysis. Subsequently, DNA was extracted using the Invisorb® Spin Forensic Kit (STRATEC Molecular, Germany) following the standard protocol. For *Diplostomum* metacercariae, the forward primer BD1 (5'-GTCGTAACAAGGTTTCCGTA-3') and reverse primer 4S (5'-TCTAGATGCGTTCGAA(G/A)TGTCGATG-3') (Bowles et al. 1993) were used to obtain the ITS1 partial sequence, with an annealing temperature of 58 °C (PCR details in Dudliv et al. (2024)), while the forward primer 18S9F and reverse primer 18S637R (Moszczynska et al. 2009) were employed to obtain the 18S partial sequence for the larval cestodes (Bothriocephalidae, Proteocephalidae). The PCR reaction mix contained 4 µl of extracted DNA, 0.3 µl of each primer (10 µM), 2 µl of buffer A, 0.2 µl of dNTPs (10 mM), 0.2 µl of $MgCl_2$ (25 mM), 0.5 U/µl of Taq polymerase and ddH_2O up to a total volume of 10 µl. PCR was performed using the KAPA2G Robust HotStart PCR Kit (Kapabiosystems, USA) in a Mastercycler ep gradient S thermocycler (Eppendorf, Germany) with the following temperature profile: 95 °C for 2 min, 33 cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 5 min, with a final extension at 72 °C for 5 min. All PCR products were purified using an ExoSAP-IT Kit (Affymetrix Inc., Santa Clara, USA) following the manufacturer's protocol. PCR products were commercially sequenced at Eurofins Genomics Germany GmbH. The sequences were checked and aligned using Geneious® v.9.0.5 and compared with the NCBI database using BLASTn to assess sequence similarity. Similarity 99.8–100% and 99.5–100% for ITS/18S and COI, respectively, was applied to perform species assignment. Newly-generated sequences were submitted to GenBank under the accession numbers: PV402115, PV402120-1, PV402623, PV402631 and PV402405-6.

Data analysis

Only those parasites that could be counted were used for quantitative analysis (i.e. kinetoplastids and ciliates, except *Ichthyophthirius multifiliis*, were excluded from the analysis), while all parasite taxa were included in the qualitative analysis. Non-native parasites included species co-introduced with pumpkinseed from North America (according to Hoffman (1999)) and species introduced historically to the study area from Asia by other vectors. Acquired parasites included both European (Moravec 2001) and Asian species (Wielgoss et al. 2008; Konečný et al. 2018; Ondračková et al. 2025) infecting pumpkinseed and Asian species infecting perch. Parasite load was expressed in terms of prevalence, intensity of infection and abundance, calculated following Bush et al. (1997). Mean abundance was calculated as the average number of parasites per host in each locality, intensity of infection as the average number of parasites per infected hosts and prevalence as the percentage of infected hosts. Differences between parasite communities were analysed at both

the infracommunity level (parasites found on a single host) and component community level (parasites found in a host population) (Bush et al. 1997).

Analysis at the infracommunity level was conducted using generalised linear mixed models (GLMM), which were used to compare parasite abundance (negative binomial distribution) and infracommunity species richness (Poisson distribution) between species (perch/pumpkinseed) and between type of population (allopatric/sympatric), with site as a random factor. The same approach was applied to compare the abundance and richness of acquired parasites, with the exception of comparisons between acquired parasites in sympatric and allopatric populations of perch, where the low incidence of acquired parasites made testing for differences in abundance pointless. Instead, we tested for differences in prevalence using GLMM with a Bernoulli distribution.

Differences in parasite diversity at the component community level were assessed using three diversity indices, i.e. the Shannon-Wiener index (H), which indicates average niche width; the dominance index (D), which expresses the extent to which a few species dominate the community; and the equitability index (J), which measures the evenness with which individuals are distributed amongst the taxa present (Magurran 2004). Permutation tests (1000 permutations) were used to compare diversity indices between groups (host species, allopatric/sympatric localities). All diversity analyses were performed using PAST software (PAlaeontologicalSTatistics, v.3.22; Hammer et al. (2001)).

Differences in parasite community composition between sympatric and allopatric populations in both host species were tested using permutational multivariate analysis of variance (PERMANOVA; Anderson (2001)) and visualised using non-metric multidimensional scaling (NMDS). Both NMDS and PERMANOVA were conducted separately on two distance matrices, i.e. a matrix based on Jaccard distances (binary) and one based on Bray-Curtis distances (quantitative, abundance data fourth-root transformed), with PERMANOVA based on 999 permutations. All statistical analyses were performed using R v.4.3.1 (R Core Team 2023), with the MASS (Venables and Ripley 2002), lme4 (Bates et al. 2015), lmtest (Zeileis and Hothorn 2002) and vegan (Oksanen et al. 2022) packages.

Results

Parasite community composition in native and introduced hosts

All pumpkinseed and all, but one perch were infected with at least one parasite taxon. In total, 38 parasite taxa were found, 31 infecting perch and 18 infecting pumpkinseed (Table 2). Three parasite species co-introduced with pumpkinseed from North America, the monogeneans *Onchocleidus dispar* and *O. similis* and the trematode *Posthodiplostomum centrarchi*, were found solely on their natural host, representing 85% of all pumpkinseed parasites. Three additional species, *A. crassus*, *Neoergasilus japonicus* and *Sinanodonta woodiana*, were introduced to the study area from Asia, contributing 5% and 10% to the parasite communities of perch and pumpkinseed, respectively. Trematode metacercariae dominated the parasite communities of both host species, representing 55% of metazoan parasites in perch (nine species) and 75% in pumpkinseed (four species). The maximum intensity of infection in pumpkinseed was by metacercariae of *P. centrarchidae* (up to 1111 parasites per fish), whereas in perch, it was metacercariae of *B. polymorphus* (up to 33 parasites).

Table 2. List of parasite species found in Eurasian perch *Perca fluviatilis* and pumpkinseed *Lepomis gibbosus* in lentic waterbodies in the basin of the lower Morava River.

		Perca fluviatilis				*Lepomis gibbosus*			
		ALLOPATRIC		SYMPATRIC		ALLOPATRIC		SYMPATRIC	
		Prevalence	Abundance	Prevalence	Abundance	Prevalence	Abundance	Prevalence	Abundance
Opisthoconta									
Ichthyosporea	*Dermocystidium* spp.	15.6%	0.4						
Excavata									
Kinetoplastida	Cryptobiidae spp.			3.3%				3.3%	
SAR									
Oligohymenophorea	*Apiosoma* spp.	33.3%		56.7%					
	Capriniana piscium			3.3%					
	Epistylis spp.	2.2%							
	Scyphidia spp.	15.6%		20.0%					
	Trichodinidae spp.	40.0%		63.6%		13.3%			
	Ichthyophthirius multifiliis			3.3%	0.03	15.6%	0.8		
Animalia									
Myxosporea	Myxosporidia sp. (muscle)	13.3%	0.3						
Monogenea	*Onchocleidus dispar*					57.8%	20.1		
	Onchocleidus similis					20.0%	0.6	50.0%	10.0
Trematoda	*Bucephalus polymorphus*			100%	6.5			76.7%	14.6
	Rhipidocotyle campanula	2.2%	0.02						
	Diplostomum pseudospathaceum					26.7%	0.3	3.3%	0.03
	Diplostomum sp. Lineage 3 sensu Faltýnková et al. 2022			6.7%	0.07				
	Tylodelphys clavata			46.7%	3.3			6.7%	0.13
	Clinostomum complanatum	51.1%	2.4	26.7%	0.4				
	Cyathocotyle prussica			3.3%	0.03				
	Holostephanus cobitidis			3.3%	0.03				
	Holostephanus luhei			10.0%	0.1				
	Metorchis xanthosomus			3.3%	0.03				
	Posthodiplostomum centrarchi					88.9%	131.8	16.7%	0.2
Cestoda	*Bothriocestus claviceps*							6.7%	0.1
	Proteocephalus percae	4.4%	0.1	23.3%	0.5				
	Triaenophorus nodulosus	11.1%	0.1			8.9%	0.3		
	Valipora campylancristrota							6.7%	0.1
Nematoda	*Anguillicola crassus*	4.4%	0.2	13.3%	0.4			20.0%	0.5
	Camallanus truncatus	26.7%	0.8						
	Philometra obturans	15.6%	0.5						
	Philometra sp.	2.2%	0.02						
	Schulmanela petruschewskii					8.9%	0.3	6.7%	0.07
Acanthocephala	*Acanthocephalus lucii*	11.1%	0.3	3.3%	0.03				
	Pomphorhynchus laevis			3.3%	0.3				
Bivalvia	*Sinanodonta woodiana*			23.3%	0.4	26.7%	1.2	3.3%	0.07
Crustacea	*Ergasilus sieboldi*	57.8%	1.4	50.0%	4.4			3.3%	0.03
	Neoergasilus japonicus			6.7%	0.07			60.0%	24.7
	Paraergasilus longidigitus			23.3%	0.8				
Branchiura	*Argulus foliaceus*	6.7%	0.1	3.3%	0.03	13.3%	0.2	3.3%	0.03

Nine parasite species were found in both perch and pumpkinseed hosts, i.e. the ciliates *Ichthyophthirius multifiliis*; the larval trematodes *Bucephalus polymorphus* and *Tylodelphys clavata*; the larval cestode *Triaenophorus nodulosus*; the larval nematode *A. crassus*, bivalve glochidia of *S. woodiana*, the copepods *Ergasilus sieboldi* and *N. japonicus* and the branchiuran *Argulus foliaceus* (Fig. 1). Additionally, representatives of the Trichodinidae and Cryptobiidae families were found in both hosts, although species identification was not performed (Table 2).

Parasite abundance and diversity in native and introduced hosts

Mean parasite abundance differed significantly between host species (GLMM, $Z = 5.931$, $p < 0.001$), being ten times higher in pumpkinseed (113.6 ± 233.1 S.D.) compared to perch (10.6 ± 8.8). Parasite infracommunity species richness ranged from 1–10 in perch and 1–7 in pumpkinseed, with significantly higher species richness in perch (3.9 ± 1.9) than pumpkinseed (2.8 ± 1.2) (GLMM, $Z = 3.849$, $p < 0.001$).

Parasite diversity indices differed significantly between native and non-native hosts (permutation tests, $p < 0.0001$ for all comparisons). Both the Shannon diversity index and equitability were higher in perch than pumpkinseed (Shannon diversity index: perch $H = 2.524$, pumpkinseed $H = 1.099$; equitability: perch $J = 0.735$, pumpkinseed $J = 0.380$), while dominance was lower in perch ($D = 0.118$) than pumpkinseed ($D = 0.509$).

Allopatric vs. sympatric populations

Parasite abundance was significantly higher in sympatric than allopatric perch populations (GLMM: $Z = 4.081$, $p < 0.001$), but did not differ in pumpkinseed populations (GLMM, $Z = 0.195$, $p = 0.846$; Fig. 2A). Similarly, infracommunity richness was significantly higher in sympatric than allopatric perch populations (GLMM, $Z = 3.206$, $p < 0.001$), while no differences were found in pumpkinseed populations (GLMM, $Z = 0.174$, $p = 0.862$; Fig. 2B). However, sympatric pumpkinseed populations were parasitised by a significantly higher abundance of acquired parasites (including both European and Asian species) than allopatric populations (GLMM, $Z = 2.864$, $p = 0.004$). Perch from sympatric populations hosted acquired parasites significantly more often than those from allopatric populations (GLMM, df = 1, 73, P = 0.001).

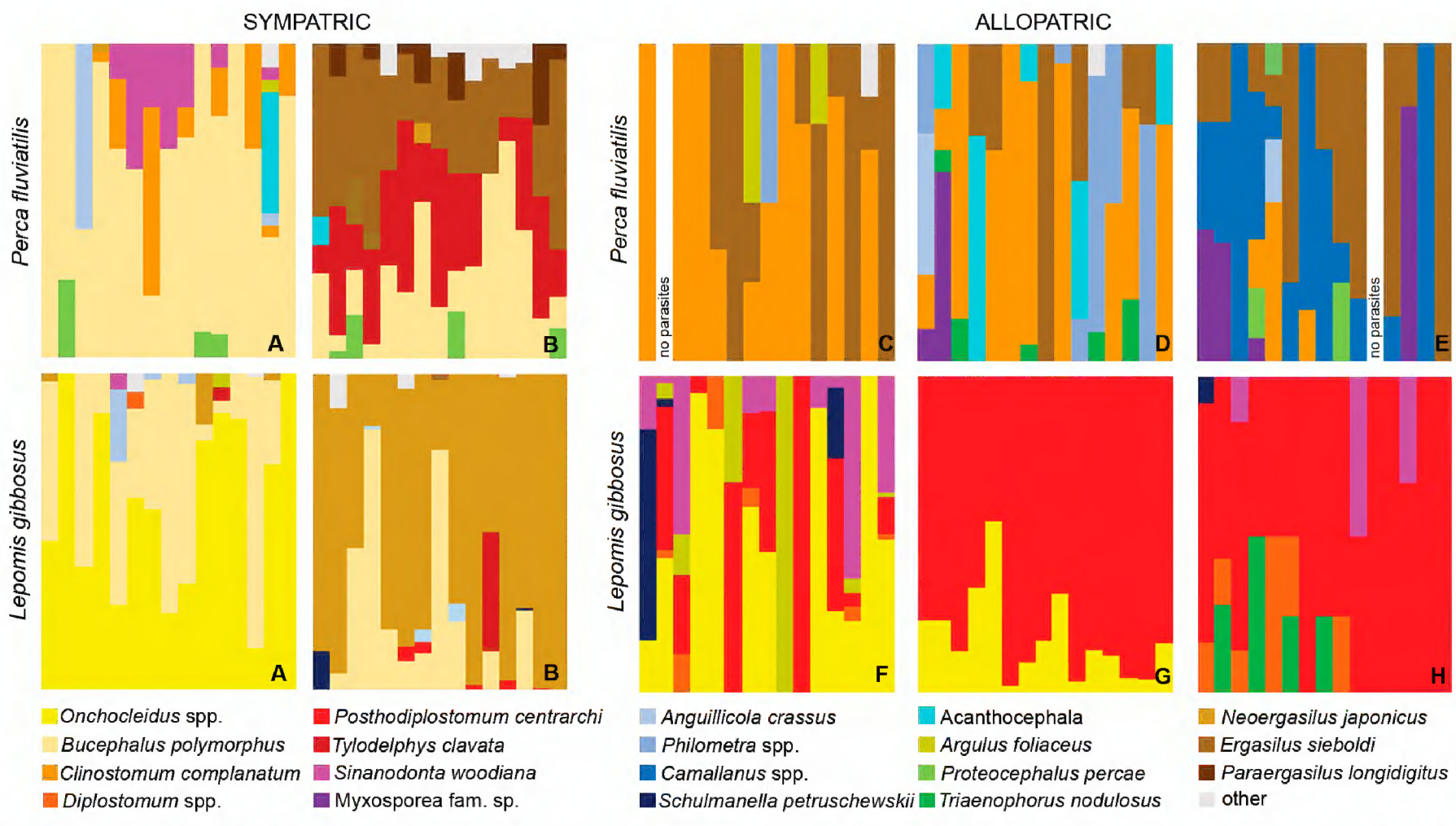

Figure 1. Proportion of metazoan parasite taxa in infracommunities of native European perch *Perca fluviatilis* and non-native pumpkinseed *Lepomis gibbosus* examined at eight localities: 1) with sympatric occurrence of perch and pumpkinseed: A = U Jezu, B = Babice; 2) with allopatric perch: C = Dedava, D = Kanada, E = Penne; and 3) with allopatric pumpkinseed: F = Pohansko, G = Oxbow D3, H = Oxbow D6.

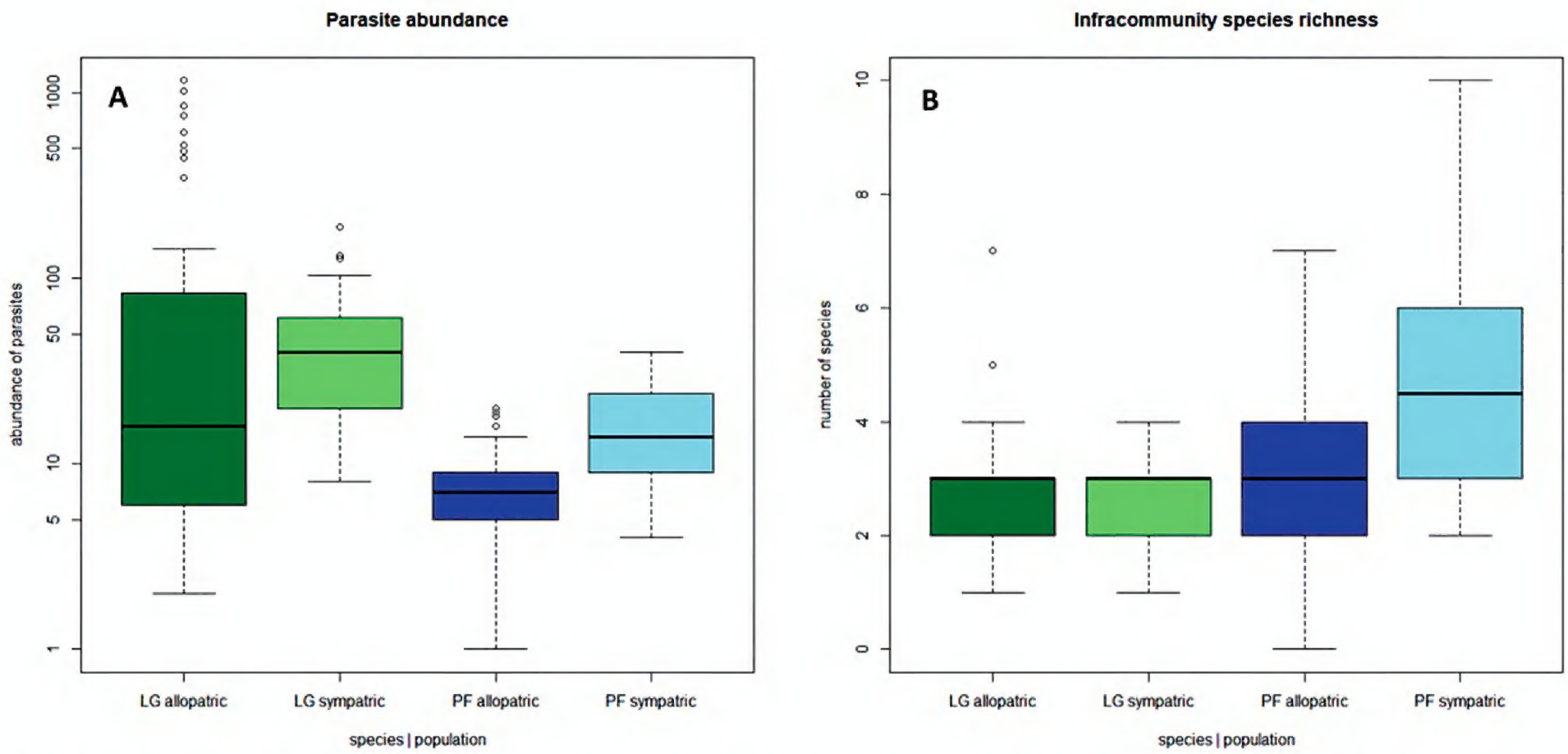

Figure 2. Parasite abundance (**A**) and infracommunity species richness (**B**) in sympatric and allopatric populations of introduced pumpkinseed *Lepomis gibbosus* (green bars) and native European perch *Perca fluviatilis* (blue bars) in small lentic waterbodies in the lower Morava River Basin. Parasite abundance is expressed with a logarithmic scale. Horizontal line: median, box: interquartile range, whiskers: non-outlier range, points: outliers.

Parasite species richness was higher in sympatric than allopatric populations of both species (18 and 13 in perch, 13 and 9 in pumpkinseed), despite just two sympatric sites being compared to three allopatric sites. While Shannon diversity did not differ in perch (H = 1.905 and 1.781 for allopatric and sympatric populations), it was significantly higher in sympatric (H = 1.173) compared with allopatric (H = 0.527) pumpkinseed populations. In pumpkinseed, equitability was higher in sympatric (J = 0.458) compared to allopatric populations (J = 0.240), while dominance was higher in allopatric populations (D = 0.736) compared to sympatric (D = 0.361; $p < 0.001$ for all comparisons). In contrast, significantly lower dominance (D = 0.210; $p = 0.01$) and higher equitability (J = 0.743; $p < 0.001$) were observed in allopatric populations compared to sympatric ones in perch (D = 0.244; J = 0.616).

The composition of parasite communities in both perch and pumpkinseed differed significantly between sympatric and allopatric populations. This was confirmed for both Jaccard (Fig. 3A) and Bray-Curtis (Fig. 3B) distance matrices (PERMANOVA, df = 1, 73, P = 0.001 for all analyses).

Discussion

In this study, we tested whether the introduction of a competent non-native fish host, the pumpkinseed, might shift the parasite load and community composition of native fish species, such as the Eurasian perch. We found that the parasite communities of native perch co-occurring with non-native pumpkinseed at sympatric sites differed significantly from those of perch at allopatric sites, which were not influenced by the introduction of pumpkinseed. Specifically, parasite communities of perch populations living in sympatry with pumpkinseed exhibited significantly higher parasite abundance, infracommunity richness and dominance, along with lower equitability compared to those in allopatric populations. These findings suggest that the introduction of pumpkinseed may have contributed to shifts in parasite abundance, diversity and community composition in the native fish species.

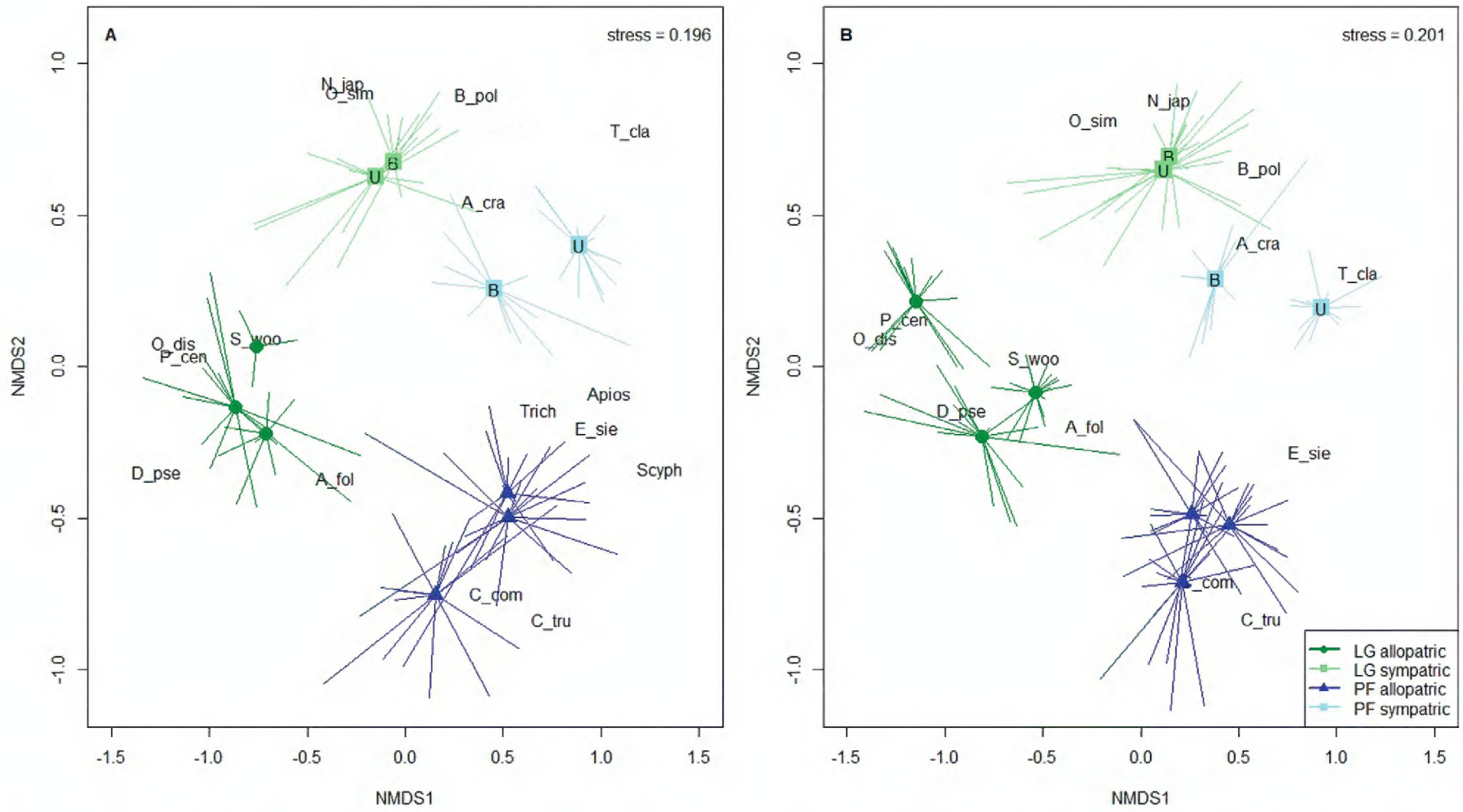

Figure 3. Jaccard (**A**) and Bray-Curtis (**B**) similarity in parasite assemblage structure in allopatric and sympatric populations of native European perch (*Perca fluviatilis*; PF) and non-native pumpkinseed (*Lepomis gibbosus*; LG), projected in a two-dimensional space using non-metric multidimensional scaling. Lines connect positions of each infracommunity to a centroid of respective site (in sympatric populations, each site is marked by a letter: B = Babice, U = U Jezu. Parasite abbreviations: A_cra – *Anguillicola crassus*; A_fol – *Argulus foliaceus*; Apios – *Apiosoma* spp.; B_pol – *Bucephalus polymorphus*; C_com – *Clinostomum complanatum*; C_tru – *Camallanus truncatus*; D_pse *Diplostomum pseudospathaceum*; E_sie – *Ergasilus sieboldi*; N_jap – *Neoergasilus japonicus*; O_dis – *Onchocleidus dispar*; O_sim – *Onchocleidus similis*; P_cent – *Posthodiplostomum centrarchi*; Scyph – *Scyphidium* spp.; S_woo – *Sinanodonta woodiana*; T_cla – *Tylodelphys clavata*; Trich – Trichodinidae.

Parasite abundance in sympatric vs. allopatric perch populations

Perch from sympatric sites had a higher mean parasite abundance compared to those from allopatric sites, a trend not observed in pumpkinseed, where parasite abundance varied widely across sites. The increased abundance in sympatric perch populations was primarily driven by parasitisation by *B. polymorphus* and *T. clavata*, both of which also frequently parasitise pumpkinseed. Pumpkinseed appears to be highly susceptible to *B. polymorphus* metacercariae, which encyst in the host's fins and muscles. Previous studies have noted the high susceptibility of non-native Ponto-Caspian gobies to *B. polymorphus* in the lower Morava River (Ondračková et al. 2015a), the Vistula River (Kvach and Mierzejewska 2011) and the middle Danube (Mühlegger et al. 2012). Invasion of the lower Morava River by gobies, along with the dispersal of *Dreissena polymorpha*, the intermediate host of *B. polymorphus*, led to intense infection rates in many local fish species (Ondračková et al. 2015a), indicating a possible case of parasite spill-back.

A similar trend may be emerging in this study, with elevated infection intensities in pumpkinseed possibly contributing to an increased abundance of *B. polymorphus* in the environment, which, in turn, may have heightened infection rates in native species like perch. As a generalist parasite at the second intermediate host level (fish), *B. polymorphus* appears capable of infecting a range of non-native species, including pumpkinseed, that are not adapted to the parasite. The high prevalence (100%) and abundance of this parasite in sympatric perch populations is surprising as percids, including perch, are not typically preferred intermediate

hosts for *B. polymorphus* (Moravec 2001). This observation may thus be linked to the presence of competent pumpkinseed hosts at these localities.

In contrast to *B. polymorphus*, we were unable to assess the specific contribution of pumpkinseed to dynamics of the eye-fluke *T. clavata* in the study area. The metacercariae of *T. clavata* are generalists, capable of infecting a wide range of fish species (WoRMS 2025); nevertheless, perch commonly serve as hosts for this parasite, often showing high infection intensities (Kennedy 2001; Morozińska-Gogol 2013; Vivas Muñoz et al. 2017). Though pumpkinseed appear to be suitable hosts for *T. clavata*, as observed in various European regions (Piasecki and Falandysz 1994; Ondračková et al. 2021a; Innal and Stavrescu-Bedivan 2022; Kvach et al. 2023), its actual role in the parasite's dynamics is likely to be relatively low. One reason for this may be the low host specificity of *T. clavata* at the fish intermediate host level, suggesting that newly-introduced hosts may simply replace another susceptible host within the community. Furthermore, infection levels in pumpkinseed were not particularly high. This highlights the importance of accounting for the traits of parasite species when considering the potential spill-back of local parasites associated with the introduction of non-native hosts.

Factors influencing diversity and composition of perch parasite communities

Native perch from small lentic waterbodies in the lower Morava River exhibited a diverse range of parasite species, surpassing the parasite diversity found in studies with similar sampling efforts in other regions, such as Poland (Sobecka and Słomińska 2007; Morozińska-Gogol 2013; Bielat et al. 2015), Germany (Ondračková et al. 2015b; Suthar et al. 2022) or Bulgaria (Kuzmanova et al. 2019). In particular, perch from sympatric sites (co-occurring with pumpkinseed) exhibited higher parasite species richness than allopatric perch, with levels similar to those in perch from larger waterbodies sampled over multiple seasons (Halmetoja et al. 2000; Valtonen et al. 2003; Wierzbicka et al. 2005; Shukerova et al. 2010). It is important to note, however, that the high parasite diversity in sympatric perch populations cannot be solely attributed to the introduction of pumpkinseed. Several native digenean metacercariae that were rarely found in perch and absent in pumpkinseed, suggest that other environmental and ecological factors may have influenced composition of the perch parasite community.

A wide range of biotic and environmental factors contribute to the structure and species composition of parasite communities. By comparing parasite communities in sympatric and allopatric perch populations, we aimed to minimise the influence of host traits such as diet, body size, longevity and reproductive behaviour (Sasal et al. 1997; Poulin 2008). Nonetheless, habitat-related factors cannot be overlooked. To limit environmental variability, the fish were sampled from small, semi-natural lentic waterbodies in the lower Morava River Basin, where fish communities are predominantly composed of phytophilic and phyto-lithophilic species (Bartošová et al. 2001; Kvach et al. 2021b). Moreover, all study sites were easily accessible to piscivorous birds, which serve as potential definitive hosts for a range of parasite species. The sites were surrounded by trees and had a certain amount of vegetation, providing favourable conditions for the presence of first intermediate hosts of trematodes. Despite this, we cannot rule out the influence of environment on parasite community composition in perch, especially in the case of species that do not use pumpkinseed as their host.

Given the ecological (Fobert et al. 2011) and, to some extent, phylogenetic (Series Eupercaria; Betancur-R et al. (2017)) relatedness between Eurasian perch and pumpkinseed, we anticipated a shift of perch parasites towards pumpkinseed in sympatric sites, as found in the abundance of acquired parasites in our study. Furthermore, occurrence of the copepod *E. sieboldi* and metacercariae of *T. clavata* in pumpkinseed appears to be directly associated with perch co-occurrence, as both parasites, while generalists, are particularly common in Eurasian perch (Lucký and Navrátil 1984; Wierzbicka et al. 2005; Morozińska-Gogol 2013; Kvach et al. 2023). Thus, the sympatric occurrence of pumpkinseed with perch appears to contribute to the increased abundance of potentially competent parasites, able to parasitise both species.

Co-invading parasites and spill-over effects

Parasites that co-occur with their original host species during introductions, often switch to local species after successfully establishing in the new environment, effectively making these parasites co-invaders (Lymbery et al. 2014). In this study, three such co-invading parasites were found to parasitise both host species, the Asian *A. crassus* (a nematode), *N. japonicus* (a parasitic copepod) and *S. woodiana* (an Asian freshwater mussel). Interestingly, these parasites tend to infect invasive species more frequently than native species, often reaching higher infection levels in the former.

The nematode *A. crassus*, introduced into Europe in the 1980s, parasitises the swim bladder of eel (*Anguilla anguilla*; Wielgoss et al. (2008)) and uses a range of fish species as paratenic hosts. Interestingly, non-native fish species often exhibit high prevalence and abundance of *A. crassus* larvae, as found for pumpkinseed and brown bullhead (*Ameiurus nebulosus*) in the Albert Canal in Belgium (Thomas and Ollevier 1992) and for pumpkinseed, monkey goby (*Neogobius fluviatilis*) and topmouth gudgeon (*Pseudorasbora parva*) in Lake Balaton in Hungary (Székely 1994). In contrast, native species, such as ruffe (*Gymnocephalus cernuus*), have lower infection rates (Thomas and Ollevier 1992), while native cyprinids exhibit a strong host reaction against the parasites, with the majority of larvae found dead (Székely 1994). In our study, while prevalence in pumpkinseed was lower than that reported in Belgium or Hungary, all parasites observed were alive, suggesting that the introduction of pumpkinseed into the ecosystem may have played a role in the higher prevalence of this parasite at sympatric localities. Furthermore, the increased prevalence of *A. crassus* larvae in sympatric perch suggests that inclusion of infected pumpkinseed in the diet of eel, the definitive host of *A. crassus*, may have contributed to a rise in parasite abundance in the region, with consequent effects on infection of native fish species.

The parasitic copepod *N. japonicus*, native to East Asia, has rapidly spread worldwide over the past half-century and is now considered a successful invasive parasite (Ondračková et al. 2025). In our study area, the occurrence of *N. japonicus* was closely linked to the introduction of pumpkinseed. An abundant population of *N. japonicus* occurs in pumpkinseed at the nearby Tovačov sandpits (Ondračková et al. 2021a), from where several pumpkinseed specimens were illegally introduced to the Babice oxbow (M. Býček, personal communication). Notably, *N. japonicus* were absent at another nearby oxbow where pumpkinseed had not been introduced (personal observation). Over the past decade, *N. japonicus* appear to have switched to infecting other fish species, including perch, as in the current study. Thus, the sympatric presence of pumpkinseed facilitated the invasion success of *N. japonicus*, representing a case

of invasional meltdown and spill-over, as the parasite was apparently co-introduced with pumpkinseed, from which it switched to other local fish species.

The Asian freshwater mussel *S. woodiana*, present in Europe since 1979, has rapidly expanded its non-native range over recent decades (Konečný et al. 2018). The parasitic larvae, glochidia, can infect a range of fish species, including both co-invasive and native species (Douda et al. 2012). In Czech lowland rivers, non-native Ponto-Caspian gobies have been identified as important vectors for this mussel, providing another example of an invasional meltdown (Šlapanský et al. 2016). Though the history of *S. woodiana* invasion in the study area is not fully documented, it was first recorded in 1996 in an oxbow of the lower Dyje River, from where it spread throughout the region (Beran 2008). Currently, *S. woodiana* is common at many localities in the lower Morava River Basin, including the river itself and adjacent lentic waterbodies (Ondračková et al. 2019b). While previous European studies have occasionally reported *S. woodiana* infecting perch and pumpkinseed (Huber and Geist 2019; Ondračková et al. 2023), our study confirms that both species are susceptible to infection. It is important to note, however, that the success of parasite metamorphosis was not assessed in this study. Nevertheless, in an experimental study, Huber and Geist (2019) found that infected perch exhibited high glochidia loss during metamorphosis, which may limit the invasive mussel's success. The developmental success of *S. woodiana* remains unknown in pumpkinseed and further research is needed to assess the competence of pumpkinseed as a host for this invasive mussel species.

Parasite load in non-native pumpkinseed

The introduction of species into new environments is often accompanied by a reduction in their natural enemies, including parasites, which can provide the introduced species with a competitive advantage over native species. Indeed, a reduction in parasitisation, both in terms of species richness and prevalence, is commonly observed when comparing native and non-native species across their respective ranges (Torchin et al. 2003). Such reductions are also often evident when comparing introduced and native species, with parasite species richness and prevalence tending to be lower in introduced species compared to their sympatric native hosts (Gendron et al. 2012; Ondračková et al. 2021b). In the case of non-native pumpkinseed, parasite prevalence was found to be comparable to that of native perch; however, species richness at both the component community and infracommunity levels was generally lower in pumpkinseed populations. This trend aligns with findings from other invaded regions across Europe (e.g. Stoyanov et al. (2017a); Ondračková et al. (2021a, 2023); Kvach et al. (2023, 2024); Dudliv et al. (2024)).

Changes in parasite abundance between native and introduced host populations are often highly variable, with some introduced hosts experiencing lower, equal or higher parasite abundance (e.g. Francová et al. (2011); Kołodziej-Sobocińska et al. (2018)). The wide variation in parasite abundance and prevalence in non-native pumpkinseed populations across Europe reflects several contributing factors, including genetic background, associated with a possible bottleneck effect (Ondračková et al. 2021a); feeding behaviour, linked to intermediate host availability (Hnilička et al. 2024); and the absence of other local fish species that could act as a source of parasites for the invader (Ondračková et al. 2019a). This study also observed significant variation in the parasite abundance of pumpkinseed populations, with high numbers of co-introduced North American parasites, par-

ticularly metacercariae of *P. centrarchi*, a parasite that has only recently been discovered in Europe (Kvach et al. 2017; Stoyanov et al. 2017b). Despite the lower species richness of the pumpkinseed parasite community, the high abundance of these co-introduced parasites could limit the invasiveness of pumpkinseed to some degree, at least in the lower Morava River region, where both monogenean and digenean parasites of centrarchid fishes have successfully established.

Conclusions

Our study demonstrated a change in parasite abundance, species richness and community composition in a native fish species, the Eurasian perch, at localities where the fish lived in sympatry with introduced pumpkinseed. Despite a low number of sampling localities, the main limitation to this study, our data clearly show a significant difference in parasite community structure between sympatric and allopatric perch populations. At sympatric sites, perch hosted parasite communities with higher abundance and richness, suggesting that the presence of the non-native fish host contributed to a shift in the parasite load of native perch populations. These changes were not observed at allopatric sites, where native perch hosted relatively stable and undisturbed parasite communities. In contrast, all parasite infection parameters (abundance, richness and community composition) in non-native pumpkinseed populations varied greatly across sites, likely reflecting the accidental acquisition of local parasites at different locations. Notably, our study indicated potential parasite spillback from pumpkinseed, particularly in relation to its high susceptibility to *B. polymorphus* and spill-over effects caused by the co-introduction of the Asian parasitic copepod *N. japonicus*, which switched from pumpkinseed to perch in the study area. Overall, our findings highlight the significant role that non-native fish species may play in altering local parasite communities, both through direct interactions with the introduced host and through their involvement in the life cycles of invasive parasites. These interactions underscore the importance of considering the broader ecological consequences of species introductions, particularly in relation to parasite dynamics.

Acknowledgements

We thank our colleagues from the Institute of Vertebrate Biology, Czech Academy of Sciences, who helped during the fieldwork. We are also grateful to representatives of the Angling clubs in Spytihnev and Babice and Lesy ČR (Czech Forestry), for the opportunity to sample in their waters. Finally, we would like to thank Dr. Kevin Roche for proofreading the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

This research was undertaken in line with the ethical requirements of the Czech Republic. Methods of sampling and euthanisation of fish used in the study were conducted within the legal requirements of the Czech Republic (§ 7 law No. 114/1992 on the Protection of Nature and Landscape).

Funding

This study was supported through the Czech Science Foundation, project No. 23-07185S.

Author contributions

Maria Yu. Tkachenko: Investigation, Writing - review and editing; Michal Hnilička: Formal analysis, Writing - review and editing; Michal Janáč: Formal analysis, Writing - review and editing; Yuriy Kvach: Writing – original draft, Writing - review and editing; Lukáš Vetešník: Investigation, Writing - review and editing; Markéta Ondračková: Conceptualisation, Data curation, Formal analysis, Writing – original draft, Writing - review and editing, Visualisation, Funding acquisition.

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Data availability

All of the data that support the findings of this study are available in the main text.

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